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Detection of a possible *trans*-splicing intermediate in *Trypanosoma brucei*

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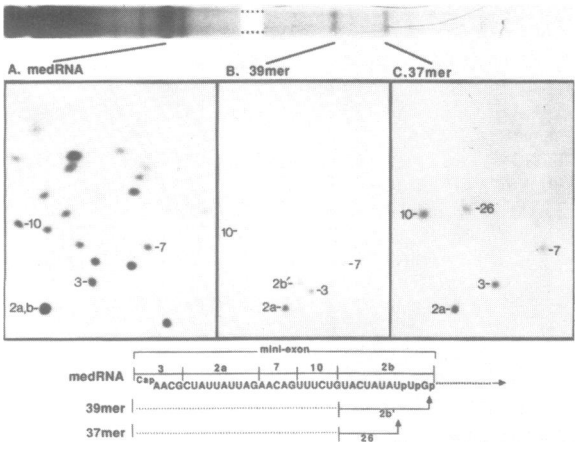
Biosynthesis of mRNA in parasitic Kinetoplastidae occurs via the joining of RNAs transcribed from unlinked genes[1]. In *Trypanosoma brucei*, a 39-nucleotide[2,3] noncoding leader, called the mini-exon, is found at the 5' end of most, probably all, mRNAs[1]. However, DNA encoding the mini-exon is unlinked to structural genes. The mini-exon is donated to mRNA from a 140-nucleotide precursor RNA, known as medRNA, which contains the mini-exon at its 5' end. The results of experiments in which the 3' portion of medRNA was released from poly (A^+) RNA by a debranching enzyme[4,5] support a trans-splicing model, mechanistically similar to cis-splicing[6], for the joining reaction. In previous work, while characterizing *in vivo* ^{32}P-labeled mini-exon-specific RNA from *T. brucei*, a 37-nucleotide RNA was detected[2]. It consisted of a version of the mini-exon lacking 2 nucleotides from its 3' end[2]. The characterization of a molecule that consists precisely of the free mini-exon, a 39mer, is presented here.

Late log phase procyclic form trypanosomes were labeled for 2 hours (in contrast to 20 hours in ref. 2) with $^{32}PO_4$. RNA was isolated, hybrid-selected with mini-exon genomic DNA and fractionated on polyacrylamide gels. Specific RNAs were eluted, fingerprinted and sequenced using analytical RNase digests, as described previously[2].



Samples from 10% polyacrylamide gels of mini-exon selected RNA are shown at top of the figure; the origin is at left. Panels A-C show RNase T1 fingerprints of the indicated RNAs; the origin of the fingerprints is at the lower right. (Panels A and C, presented for comparison, were published previously[2].) The mini-exon-specific oligonucleotides from medRNA (Panel A), spots 2(a and b), 3, 7 and 10, are indicated[2]. Fingerprints of the 39mer, in which spot 2b' replaces spot 2b; and the 37mer[2], in which spot 26 replaces spot 2b, are shown in Panels B and C, respectively. Spot 2b' was sequenced as described previously[2] and found to be identical to spot 2b, except that it lacked the 3' PO_4 that is generated by the RNase T1 cleavage of medRNA (data not shown). The alignment of the sequences is shown at the bottom of the figure.

These results indicate that the 39mer consists precisely of the free mini-exon, terminating with a 3' OH. During cleavage and branch formation in cis-splicing, the 5' exon with a 3' OH is a free intermediate[6]. The free 39mer (structurally analogous to a free 5' exon in cis-splicing) is thus a candidate trans-splicing intermediate, possibly generated by cleavage of medRNA during branch formation.

References

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